Waive and Replace the Rabbit Pyrogen Test in Lifecycle Vaccine Release

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Disclosure

Shahjahan SHAID is an employee of the GSK group of companies.

This work was sponsored by GlaxoSmithKline Biologicals SA.

GSK is committed to the replacement, reduction and refinement of animal studies (3Rs). Non-animal models and alternative technologies are part of our strategy and employed where possible. When animals are required, application of robust study design principles and peer review minimises animal use, reduces harm and improves benefit in studies.

Agenda

- Why is GSK relying on Animal Testing and the commitment to 3R?
- Achievement and Future 3R challenge on the Rabbit Pyrogen Test
- Take home message

Why is GSK relying on animal testing and the commitment to 3Rs

A small, vital and crucial part of the business

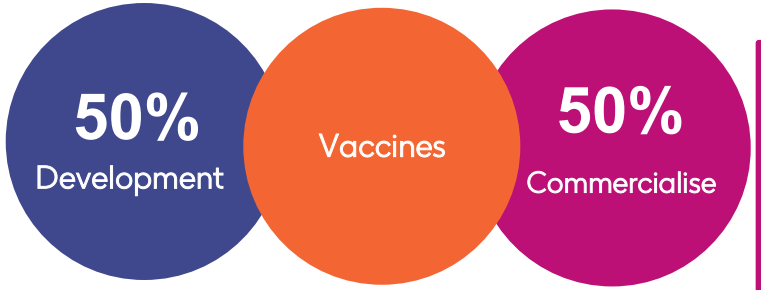
Why is GSK relying on Animal Testing

Animal Assays are required by Compendia for lifecycle vaccines

Laboratory animals used in Vaccines

R&D:

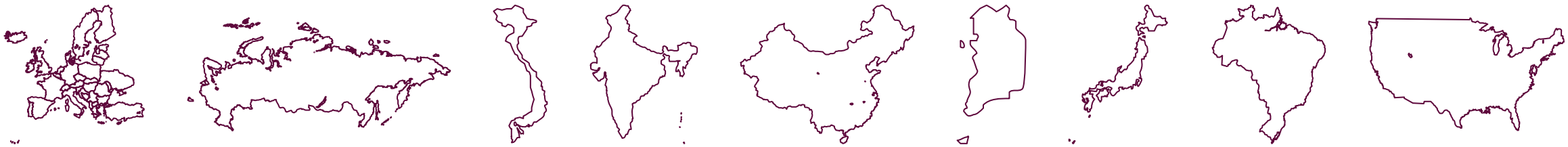
- Basic research on disease processes
- Use of models of diseases to test candidate
- Preclinical safety, efficacy, stability testings



QC:

- Animal Testing in QC mainly for Release of Vaccines in lifecycle products
- Production and control development (process and testing validation, detoxification, inactivation, etc. …)

Animal assays are required by WHO and regional Pharmacopeias which are often **not aligned** e.g. EDQM has prioritized a substitution where scientifically possible.



The substitution in a biologics license application **might therefore be rejected** by certain countries and can take years due to number of countries involved for each product.

Impact of in vivo assays in lifecycle vaccines

Animal Assays have a disproportionately high impact on the release compared to other tests

Release

Stability

Process Changes

Potency

- Serological
- Challenge

Safety / Purity

- General safety tests
- **Rabbit Pyrogen Test**
- Safety (detoxification, extraneous agent, etc.)

Identification

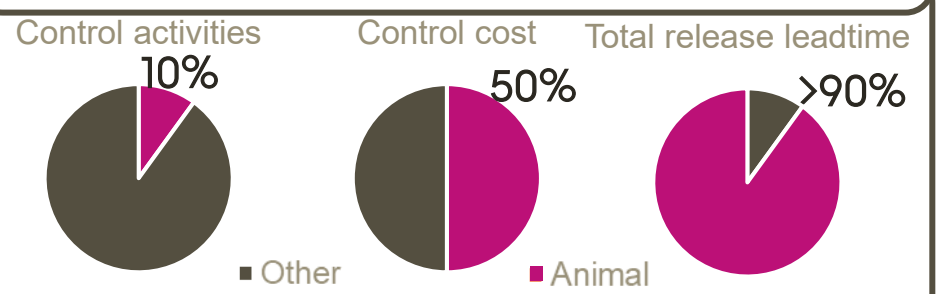
- Vaccine strain
- Antigen

Impact

Cycle time in QC testing

> 100 days

Animals used in QC testing



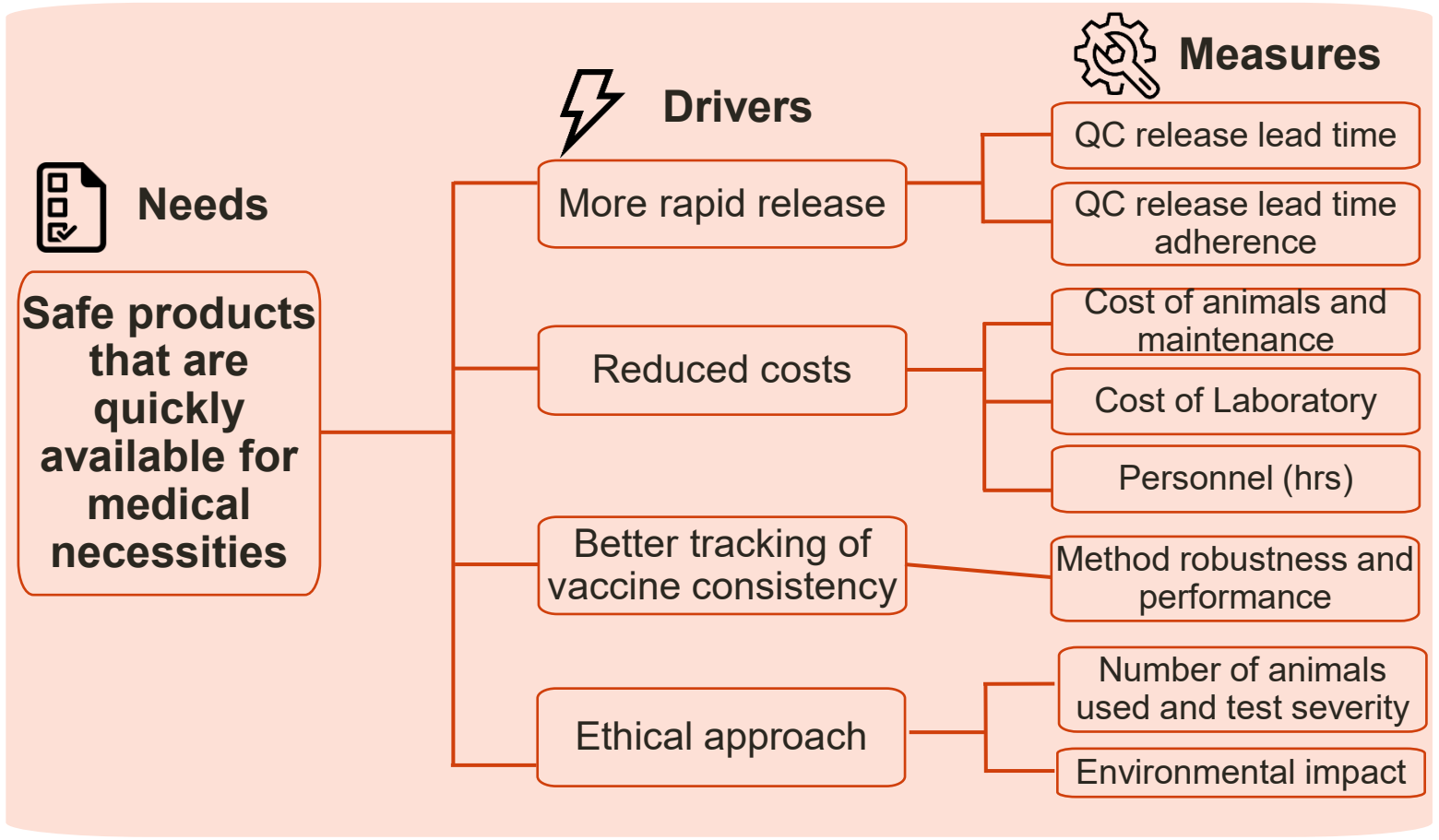
Supply impact by in vivo intrinsic variability (repeats)

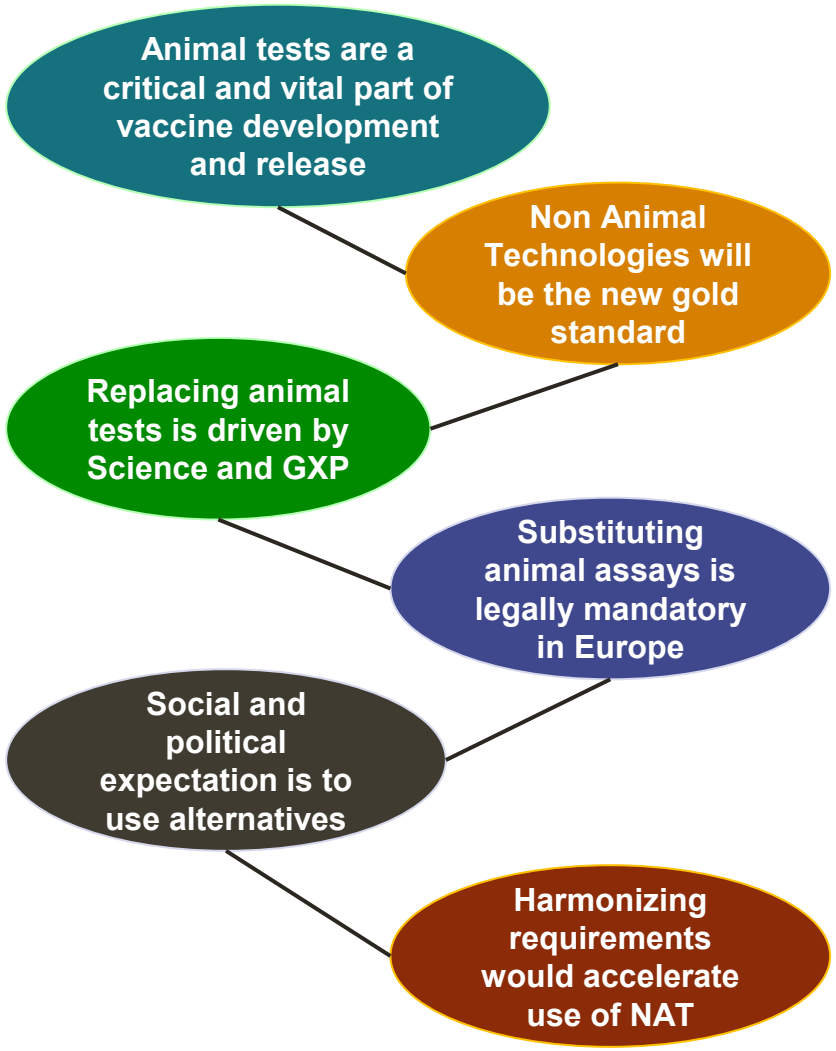
Second testing by NCL

- Animal numbers
- Lead time
- Costs

Harmonizing requirements would accelerate use of NAT

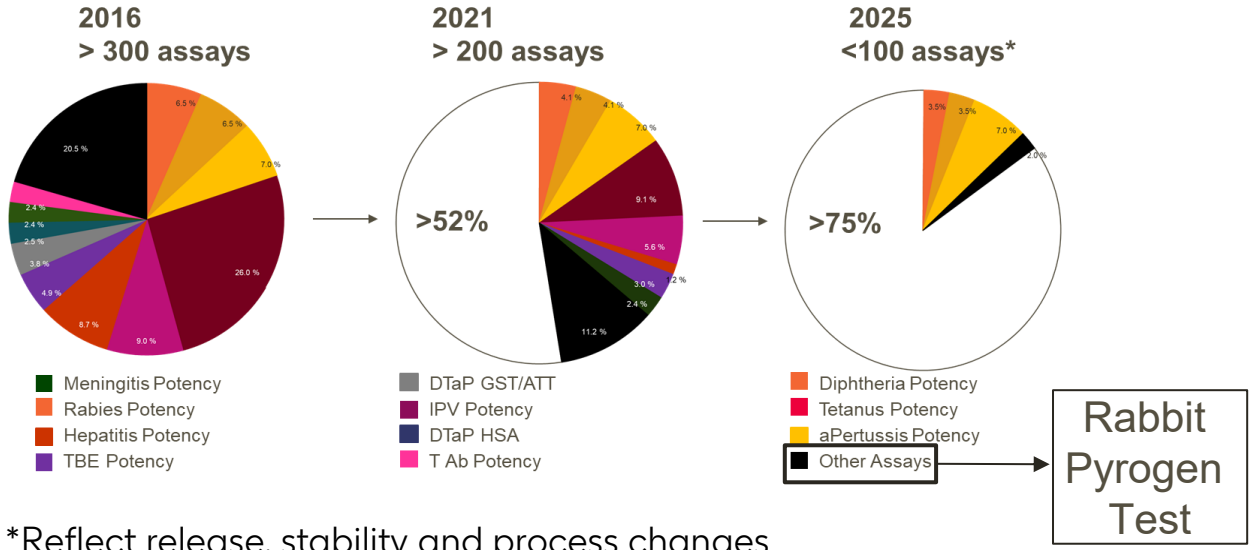
Replacing animal tests is a win win for patients and manufacturers



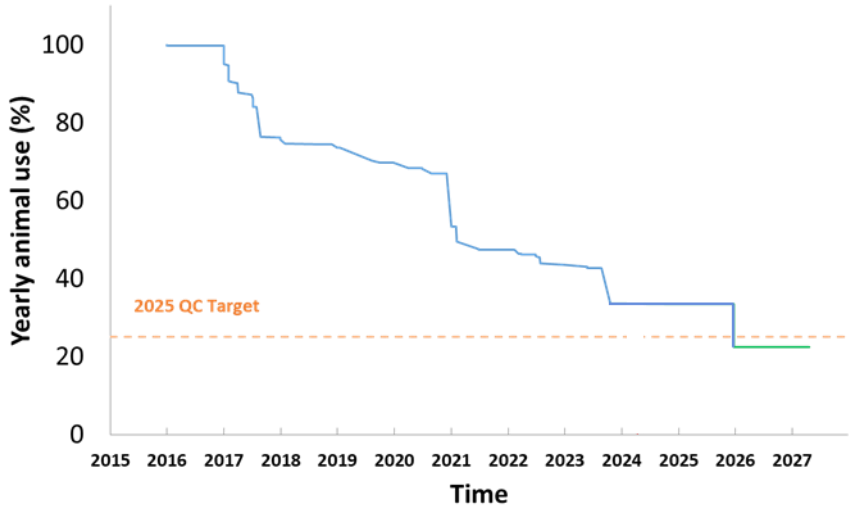


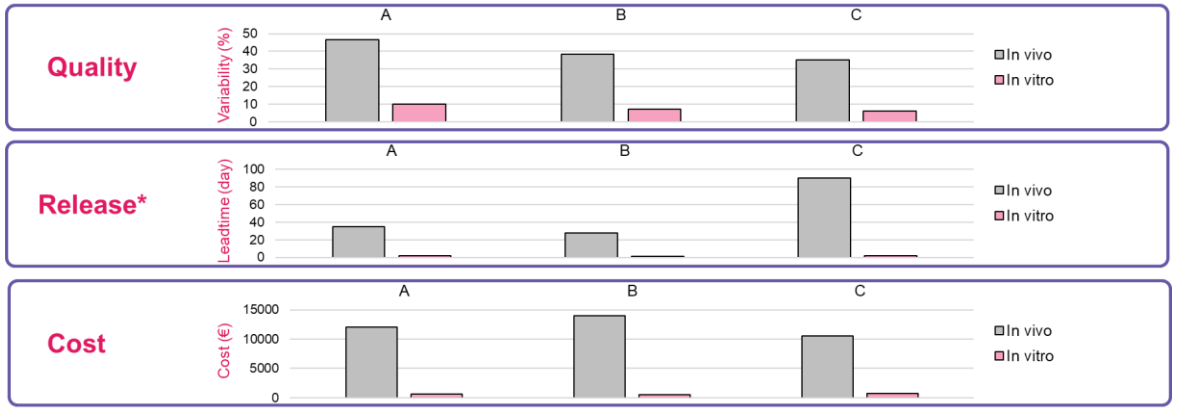
3R & GSK: Prioritize replacement to reduce animal use by 75% from 2016-2025 in QC

In vitro replacement does not jeopardize quality. Faster release; less repeats; reduced cost



*Reflect release, stability and process changes





*Difference before and after is shown

Achievement and Future 3R challenge on the Rabbit Pyrogen Test

1. Remove and rely on controls at other steps

2. Substitute by the BET test as a release

3. Replace by the MAT test as a release

Pyrogen detection is crucial and therefore mandatory to ensure patient safety

Several techniques are available allowing animal-free detection of pyrogens

Exogenous pyrogens

Non-endotoxin pyrogens

- Components from Gram-positive bacteria
- Yeast and mould
- Virus
- Particles of the environment

Endotoxins

Components from Gram-negative bacteria (LPS)

Pyrogen detection

MAT: Ph. Eur. 2.6.30

- Recognizes pyrogens with a high sensitivity.
- Established in the PEP
- Mentioned in the Ch.P and USP

RPT: Ph. Eur. 2.6.8, removal by 2026

- Measure of the **rabbit** body temperature after injection of the product.
- Former gold standard.

BET Guideline: Ph. Eur. 5.1.10

Guidelines for using the test for bacterial endotoxins (and MAT)

rFC: Ph. Eur. 2.6.32

- Non-animal-derived reagent that can detect endotoxins.
- Accepted as equivalent in the E.P.

LAL: Ph. Eur. 2.6.14

- 1st commercially available endotoxin method
- Best established and harmonized alternative to the RPT
- Broad acceptance from authorities around the world

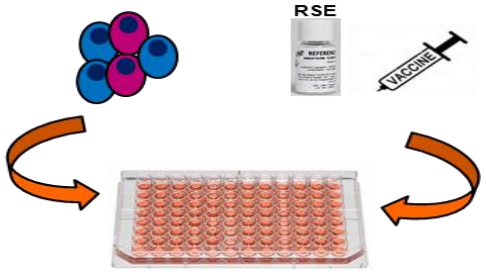
Endotoxin detection

Different Assay design of MAT

Primary Cells

Blood from healthy donors

PBMCs* isolation, qualification and cryopreservation



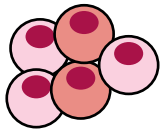
Stimulation of PBMCs with RSE** and Vaccine

Most common Readout:
IL*** detection on PBMCs supernatants by a sandwich ELISA

Data Analysis

Secondary Cells

Cell line derived macrophages i.e.THP1, NOMO-1



Permanent Cell line already qualified

Stimulation of PBMCs with RSE** and Vaccine

Several Readout:
IL by a sandwich ELISA,
IL by Fluorescence
Reporter gene by Fluorescence
Reporter gene by PCR

Data Analysis

**PBMCs= Peripheral Blood Mononuclear Cells* ***RSE = Reference Standard Endotoxin* ****IL = Interleukin i.e. IL-6, IL-1, TNF-a*

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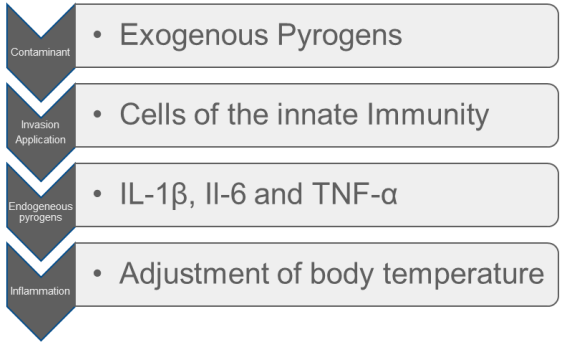
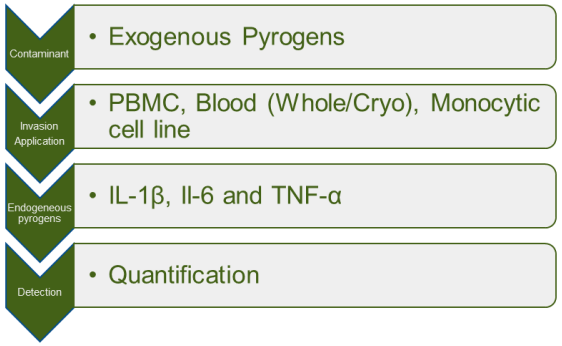
Comparison of method key characteristics of RPT and MAT

RPT is not required due to availability of Endotoxin tests and MAT

RPT



MAT



	RPT	MAT
Specificity	All rabbit pyrogens	All human pyrogens
Reagent origin	Direct animal use	Human-derived, Cell line
Availability	Since 1950s, broadly	Since 2010s, growing
Test type	Qualitative	Quantitative
Endpoint	Body temperature	Cytokine, ELISA
Duration	24h, lead time > 1week	~8h (lead time ~2days)

Endotoxin / LPS	TLR 4 has 72% homology with human on amino acid level
Single stranded RNA	TLR 7 and TLR 8 absent
DNA	TLR 9 broader recognition pattern than human

(1) doi: 10.3389/fimmu.2014.00316, (2) https://doi.org/10.1007/s00251-009-0381-z (3) https://doi.org/10.1016/j.cimid.2012.03.008

Legal requirement in different jurisdictions

Today the pyrogen methods are not aligned between the Compendia's. The trend continues. What will be the "common ground" ?

	Non-endotoxin pyrogens			Endotoxin pyrogens		
	RPT	MAT	Other methods	LAL	rFC	rLAL
E.P	Not foreseen by Jan 2026	Solemn method 2026	Not foreseen	Available	Available Equivalent method	To be determined
WHO	Available	Proposed*	Not foreseen	Foreseen	Proposed*	Proposed*
USP	Available	Discussed**	Not foreseen	Available	Available	Available
China	Available	Available	In discussion	Available	Available	To be determined
Korea	Available	Available	In discussion	Available	Available	To be determined
Japan	Available	Available	Not foreseen	Available	Alternative method	Alternative method
India	Available	Available	Not foreseen	Available	Alternative method	To be determined
Brazil	Available	Available	Not foreseen	Available	Expected	To be determined

* TRS has been published in Nov 2025

** FDA guidance on using MAT, USP Micro expert team has called for data, Europhorum

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Rabbit Pyrogen Test will be retired in the European Pharmacopoeia by 2026

Substitution by Non-animal technologies is mandatory in the EU


DIRECTIVES

DIRECTIVE 2010/63/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL

of 22 September 2010

on the protection of animals used for scientific purposes

Will be deleted from the E.P. by 2026 (incl 59 references).

According to the EU directive 2010/63 the use of non-animal technologies instead of animal tests is mandatory.

RPT will be an alternative method not described in the E.P.
Leaving the manufacturers with MAT, RFC, TAL, LAL

What are the possibilities to ensure safety and ethics without the Rabbit Pyrogen Test in QC:

1. Remove and rely on controls at other steps

2. Substitute by the BET test as a release

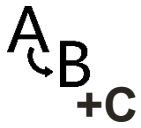
3. Replace by the MAT test as a release

Substitute the Rabbit Pyrogen Test (RPT) with a risk-based approach

Avoid test duplication and rely on the most impactful test

1. Remove and rely on controls at other steps

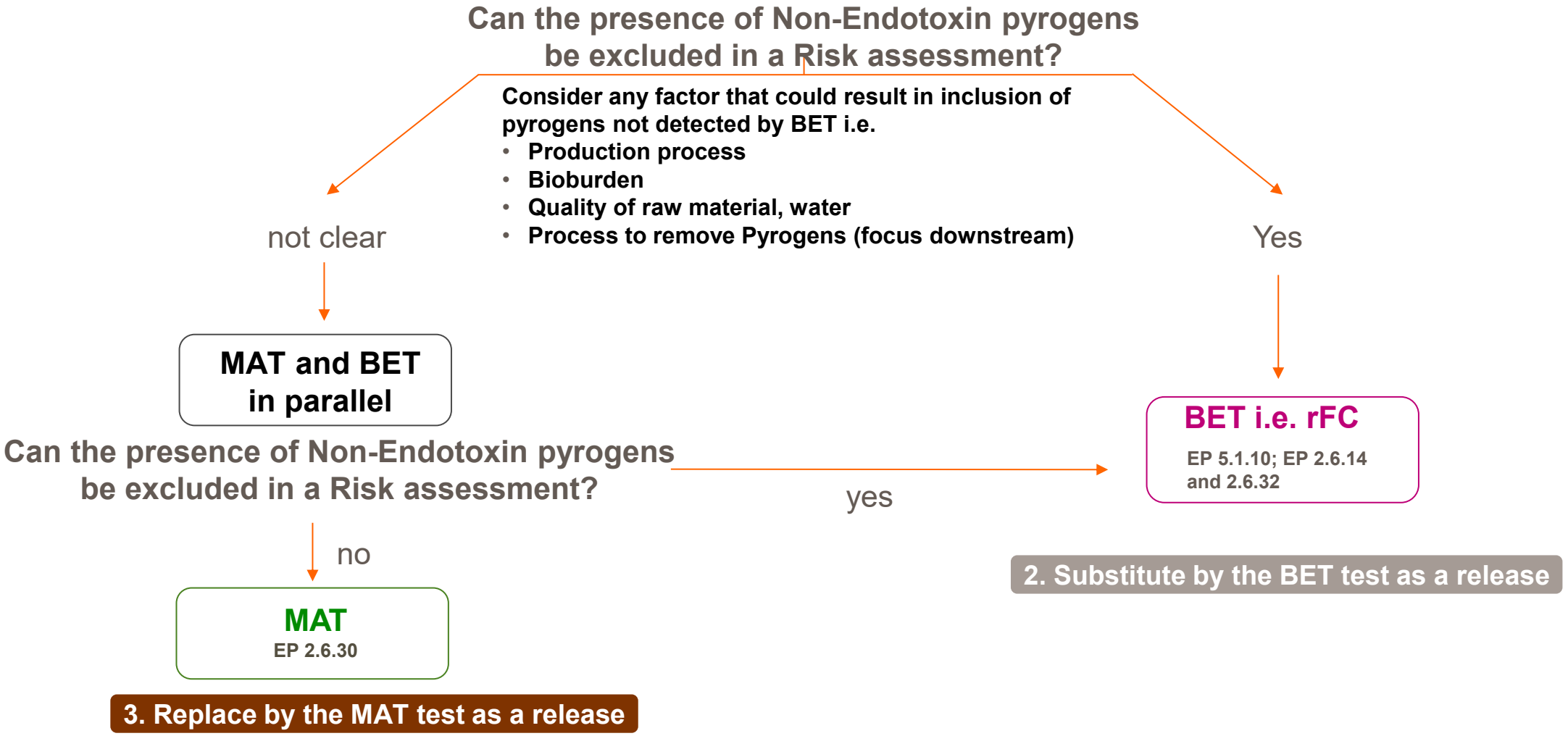
Lifecycle based on the **consistency approach**: Paradigm shift moving away from the current focus on testing **each batch as unique** with high reliance on *in vivo* models, to an **integrated in-process and final product quality monitoring** program including all available data allowing a shift to non-animal methods.
The consistency approach is based upon the principle that the **quality of a biologic is the result of the strict application of a quality system and consistent production**. Subsequent **batches are determined to be similar to clinically evaluated batches** and therefore acceptable for release through the in-process testing that comprises this quality system.



Incoming Material e.g. Antibiotics	Pyrogenicity is reduced in the manufacturing process and tested at intermediate/ Drug substance or Drug product level
	Pyrogenicity is analyzed by the Supplier and part of the Certificate of Analysis
	Pyrogenicity is evaluated at later manufacturing steps

Substitution or replacement decision depends on a risk-based approach

EP 5.1.13 describes the selection of the most adequate method



Rabbit Pyrogen Test | Monocyte Activation Test | Bacterial Endotoxin Test | recombinant Factor C | Limulus/Tachypleus Amoebocyte Lysate

Substitute the Rabbit Pyrogen Test (RPT) with a risk-based approach

The manufacturing level and overall test schedule of the product needs to be considered as well

2. Substitute by the BET test as a release

The choice of the **most appropriate** pyrogen detection strategy in **QC testing should depend on a risk-based approach** without the use of RPT. This approach should take into consideration the **lifecycle, manufacturing process, QC testing** and if applicable the **intrinsic pyrogenic characteristics of the product**.
The current GMP for human vaccines allow to assess, test and control how limited the risk is of a possible pyrogen contamination. Modern pharmaceutical manufacturers have thorough **validated controls, specifications and limits** embedded in the also **validated manufacturing process** as demanded by the **GMP rules**. Contaminants are therefore appropriately controlled.
The **absence of lipopolysaccharides** in materials or products combined with the ruling out of the presence of non-endotoxin pyrogens **is a strong indicator to justify the absence of exogenous pyrogens**.

Final Container

There is no intrinsic pyrogenicity in the product or matrix interference with the rFC/LAL/TAL test. Pyrogenicity is evaluated as a combination of the rFC/LAL/TAL and further tests e.g. bioburden sterility, environenmental monitoring

Meningoccoccal A, C, W135 Vaccine

Replace the Rabbit Pyrogen Test (RPT) with an equivalent Method

MAT recognizes pyrogens by mimicking the fever response to pyrogens in humans

3. Replace by the MAT test as a release

MAT recognizes pyrogens with a high sensitivity using **human derived monocytes** to mimic the fever response to pyrogens in vivo. Depending on the **Interleukin** (IL-6) response variability a semi quantitative or **full quantitative assay** for not inherently pyrogenic substances and a full quantitative assay for inherently pyrogenic substances are described.
Besides the technical abilities to detect all human pyrogens the MAT also allows a **validation according to today's ICH guidelines**.

The MAT is established in QC and R&D to ensure a sustainable retirement of the RPT in GSK

Final Container

Endotoxin Test is not applied and instead MAT is the method of choice due to

the intrinsic pyrogenicity compositon of the vaccine and

matrix interference with the LAL/rFC test.

Meningococcal B Vaccine

Take Home Message

Several strategies and methods are in place to stop or replace use of RPT

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2016 > 20 RPT

2021 < 10 RPT

2025 1 RPT

1. Remove and rely on controls at other steps
2. Substitute by the BET test as a release
3. Replace by the MAT test as a release

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